B-Cell Activation

Introduction

Mature naive B cells leave the bone marrow and move to secondary lymphoid organs such as the spleen, mucosa, and the follicular areas of the lymph node. Here, they're supplied with a **constant flow of antigen** from the lymph. B cells line the cortex, exposed to lymph. In the paracortex, right next to the B cells, are the T cells. In this arrangement, **naive B cells** with their IgD ("ticket to ride" and "probationary pass") are able to sample the lymph for antigen, **supervised by mature T cells**. When the naive B cell encounters an antigen, it internalizes that antigen via the immunoglobulin receptor, digests it in a lysosome, then uses MHC-2-Ag presentation to show the mature T cell what it found. If the mature T cell agrees with the B cell that what it found is foreign and worthy of reacting to, it sends growth cytokines. That B cell then proliferates, loses IgD, and undergoes isotype switching and somatic hypermutation. If the mature T cell disagrees with the B cell (such as if the B cell mistakenly presents self-antigen to the T cell), it receives only the anergy signal, losing IgM and retaining IgD.

The fate of that naive B cell that is now activated is one of two. One, to become a short-lived antibody-secreting **plasma cell**, or two, to undergo genetic variation and proliferation to remain as an immunoglobulin-as-a-surface-receptor **B memory cell**.

T-Cell-Dependent Activation

Why B cells live really close to T cells in secondary lymphoid tissue is so the adaptive immune system can exist. Up to this point in the course, the B cell immune system we described hasn't been adaptive at all. We had random generation of antigen-binding regions, silencing of binding regions that too closely identified self as foreign, and now some nonspecific activation of IgM by non-antigen polysaccharides. All this happened without any T-cell signaling, which means no memory, and no class-switching.

FROM THIS MOMENT FORWARD, we're discussing the adaptive immune system.

Imagine that an antigen flowing through the lymph node just sort of matches the B-cell antigen-binding receptor (aka the surface IgM receptor, the immunoglobulin-like Y-shaped protein on the surface of the B cell). The binding of the antigen to the receptor starts the process of T-cell-dependent B-cell activation. The B cell **endocytoses** the antigen, processes it just like a phagocyte did, produces an MHC-2 from its nucleus, and **presents the antigen** to surrounding T cells with **MHC-2-antigen**. $CD4^+$ T helper cells recognize the MHC-2-Ag complex and bind to it.

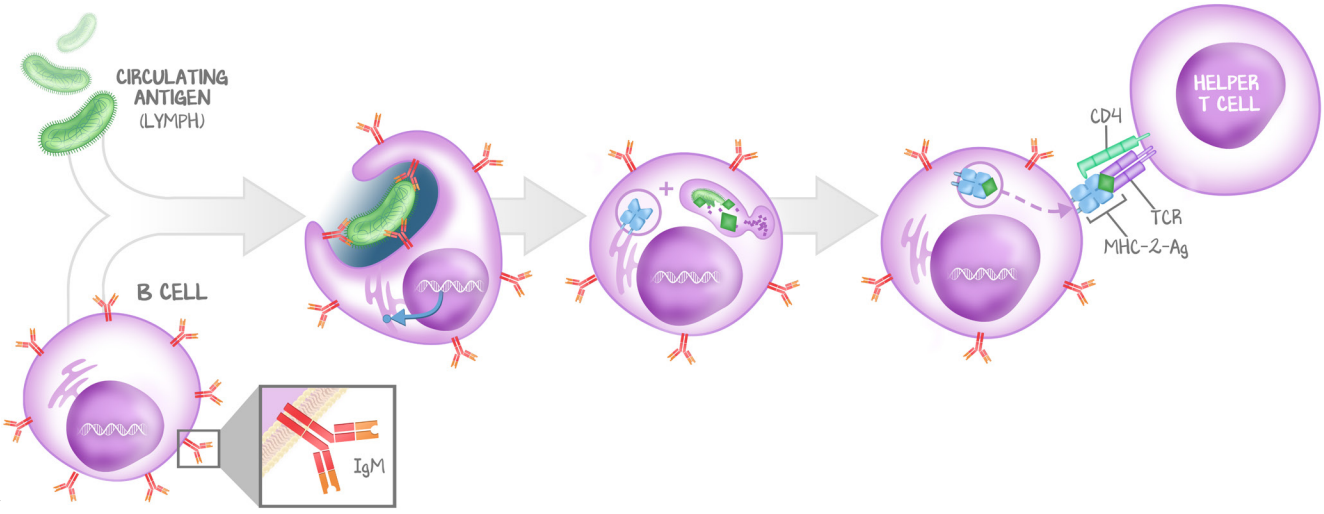

Figure 8.1: Activation of Surface Immunoglobulin
Binding of antigen to the antigen-binding domain on the immunoglobulin initiates endocytosis, with the phagosome then merging with a lysosome. The degraded products are added to MHC-2, which is then presented to the mature T cell nearby.

from being stimulated when they shouldn't. The first stimulus occurs when the **T-cell receptor** (TCR; see #9: *T-Cell Maturation* and #10: *T-Cell Activation*) binds to the **B cell's MHC-2-Antigen**, facilitated by **CD4**.

Within that same B cell, the antigen-binding receptor's binding to an antigen induces an upregulation of a surface receptor on the **B cell (B7). B7** is a surface protein that makes the B cell a more effective antigen-presenting cell. B-cell **B7** binds to the T-cell surface protein **CD28**. This stabilizes the bond between T cell and B cell. As the T cell receives the TCR signal, it upregulates T-cell **CD40-ligand**, which binds to the B-cell surface protein **CD40**, a third link that stabilizes B cell and T cell.

So to review, there are 3 connections between the B cell and T cell:
(1) TCR (T cell) with MHC-2-Antigen (on B cell, facilitated by CD4)
(2) CD28 (T cell) with B7 (B cell)
(3) CD40-L (T cell) with CD40 (B cell)

The first of these three stimuli started the process. In the presence of these **costimulatory signals** (CD40L–CD40, B7–CD28), T cells reorient their Golgi apparatus to direct a **unidirectional cytokine release from the T cells**. It's the release of **cytokines from the T cell that initiates B-cell activation**. TCR/MHC-2-Antigen, CD40L–CD40, CD28–B7 pairings all lead to cytokine release and make the process more robust. The cytokine release is necessary to initiate B-cell activation, but occurs in sufficient quantity to complete the B-cell activation only when facilitated by the costimulatory signals.

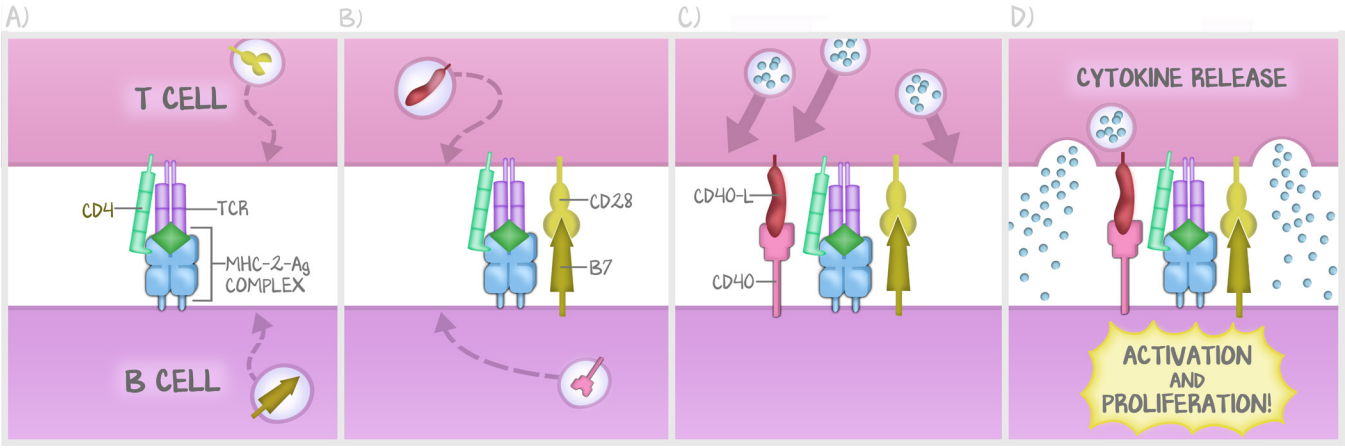

Figure 8.2: Costimulatory Signals
(a) MHC-2-Ag on the B cell reacts with the TCR on the T cell as the initial signal, stabilized by CD4. (b) The binding of TCR-MHC-2 induces the B cell to upregulate B7, which binds to CD28 on T cells. (c) The binding of TCR-MHC-2 induces also the expression of CD40L from the T cell, which binds to CD40R on the B cell. (d) With all of these costimulatory signals met, the T cell releases proliferation cytokines.

In the absence of these costimulatory signals, insufficient quantities of cytokines are released. The B cell therefore only expresses surface IgD, which results in clonal anergy.

What Activation Means

An activated B cell can differentiate into an antibody-secreting **plasma cell** or a **memory B cell.** A naive B cell expresses both IgM and IgD (IgM^+ and IgD^+). Once activated, a B cell no longer expresses IgD, and undergoes **proliferation** (clonal expansion), **isotype switching**, and **somatic hypermutation**. These processes allow the mature naive B cell to become a highly specific antigen-recognition cell called a **memory cell.** The memory B cell can remain in the germinal center of the lymphoid organ where it was activated, or it can leave and travel through the blood to other lymphoid organs. The isotype switching allows the memory B cell to secrete IgM, IgG, IgE or IgA antibodies. The somatic hypermutation allows the memory cell to be very specific to the antigen which activated the B cell to differentiate into that memory cell. Plasma cells are factories for the immunoglobulin that B cell knows how to make and become an immediate defense against the pathogen. Memory cells, on the other hand, are a more "long-term memory" of the antigen, as memory cells last for years or decades. They wait for that same antigen to return, and when it does, the response against that antigen is far more rapid and robust than the first time.

There are two waves of B cell activation.

Wave 1 of activated B cells consists strictly of **IgM-secreting plasma cells**. They leave the secondary lymphoid and go make their IgM. Having been deemed worthy of antigen-identification, the activated B cell loses expression of IgD (IgD^-), and these cells do the only thing they know how: make IgM. This IgM is not as specific as the antibody that will be produced in wave 2, but does recognize the antigen which activated the naive B cell in the first place. This IgM is **the immediate adaptive immune response to the foreign antigen.** In other words, IgM is the initial line of defense. It is not as specific as it could be, but it knows how to recognize the enemy pretty well. IgM might be enough initially in some cases. It's a powerful weapon. But the body knows it may need an even more highly trained weapon to defeat its invader, so it prepares wave 2 of activation.

Wave 2 of activated B cells stay in the lymphoid tissue and undergo **clonal expansion**, creating the **germinal center**. The clones will undergo **affinity maturation** and **isotype switching**. These cells will be turned either into **memory cells** for later, or will be turned into highly specialized snipers with immunoglobulins that are hyperspecific for this exact antigen found.

Affinity Maturation

Within a germinal center there are some B cells secreting IgM. But most cells are proliferating, and fighting each other for the privilege and signal to keep proliferating. The B cells that bind with the highest affinity for the antigen receive the growth signal. So, the better a B cell's receptor (its immunoglobulin surface receptor) binds to the antigen, the more it'll proliferate. All B cells in this germinal center start off with the same surface IgM; they all come from the one B cell that got activated. The cytokines from the T cells induce massive proliferation, which tolerates **point mutations** in coding segments of the variable regions of the antigen-binding receptors. This is called **somatic hypermutation**. Each proliferation step creates a small variation in the idiotype—either slightly higher or lower affinity for the antigen. Cells divide like crazy; the cell line explodes in numbers. But it's not true clonal expansion because of these point mutations.

Over many generations of cell divisions, those mutations with higher affinity will proliferate more and faster. This promotes more mutations, and survival (expansion) of the clones with "better" affinity. Think of it as survival of the fittest, where the "fittest" "fit" the antigen best. At the end of massive proliferation, what happens is **clonal selection**—only those cell lines with mutations that created the highest affinity for the antigen survive and multiply. At the end, the only remaining B cells are those with a **super-increased affinity** for the antigen. The only ones that survive, the only ones that are cloned, are those that matured to the point of increased affinity. This entire process is **affinity maturation**.

The B cells that are proliferating are memory cells. They have membrane-bound surface-protein immunoglobulins that induce a growth signal. The next time the antigen arises, these memory cells will immediately switch to plasma cells, without the need for somatic hypermutation or clonal expansion again. This is how the second encounter with an antigen leads to a more rapid and robust response.

Isotype Switching

Clones are proliferating. This means they're undergoing mitosis and making new proteins. IgM is that antibody that forms a pentamer when it is released into the circulation. IgM is the only thing a new B cell knows how to make. But depending on the antigen the B cell recognizes, the T cell releases certain cytokines that signal for the B cell to rearrange the encoding region on the B cell's DNA. The cytokines released will tell the B cell what isotype to create. Some of the activated B cells will continue to make IgM (they do not isotype-switch), while others will isotype-switch. The isotype-switching occurs through excision of specific portions of the B cell's DNA coding region. IgM comes first in the DNA coding region. Once the T cell tells the B cell to make a different isotype, the B cell excises the coding region for IgM, such that the **activated B cell can never make IgM again**.

So as the clones proliferate, they continue to make IgM or start making a new isotype, depending on what signal the B cell was sent by the T cell. For those B cells that undergo isotype-switching, the surface protein changes from IgM **to another isotype**, either **IgA**, **IgG**, or **IgE**.

While somatic mutations are altering the variable regions of the antigen-binding sites, increasing affinity for the antigen and promoting cell-line survival, the T cells are causing alterations to the constant regions of the heavy chain to create a new, more targeted immunoglobulin.

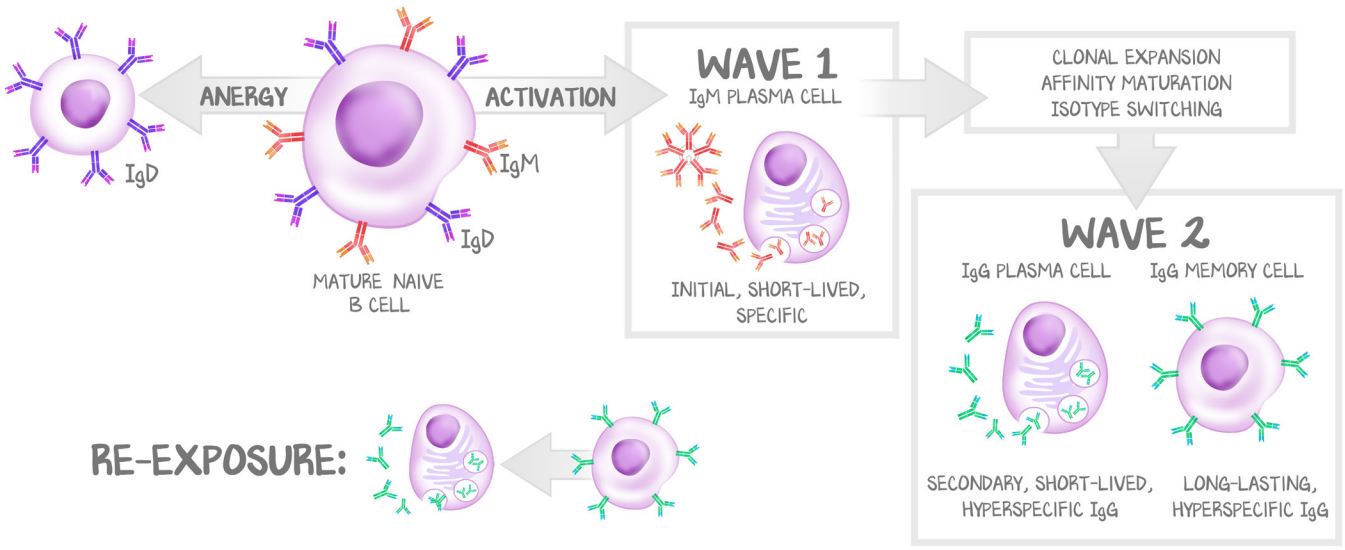

Figure 8.3: Fate of Mature Naive B Cells
A mature naive B cell presents its antigen to a veteran T cell. The mature naive B cell expresses IgM and IgD. If that T cell disapproves, that mature naive B cell is induced to anergy, expressing only IgD. If that T cell approves, that B cell gets activated. In the first wave, that B cell proliferates. Some become IgM-secreting plasma cells to fight the antigen the B cell found. Others undergo clonal expansion, affinity maturation, and isotype switching to become memory B cells, armed with hyperspecific receptors. On re-exposure, those memory cells rapidly proliferate, differentiating into IgG-secreting plasma cells. This response is robust and fast. Note that this figure uses IgG as an example for the resulting isotype. The second wave of activation can result in a plasma cell or memory cell that produces IgM, IgG, IgA, or IgE. The isotype produced by the plasma cell or memory cell will depend on the signal it received from the T cell.

Recall that when circulating immunoglobulin is not bound to a plasma membrane it is called an antibody. Circulating immunoglobulins in the body have different isotypes. Isotypes are IgG, IgM, IgA, and IgE. Each of these different isotypes behaves differently depending on the Fc portion of the antibody. IgA forms a dimer, IgG a monomer, and IgE a monomer, and IgM a pentamer. IgG, IgA, and IgE don't have the ability that IgM does to create a pentamer. IgM has a high avidity due to the combined affinity of all 5 of the antibodies that form its pentamer. IgG, IgA, and IgE don't have the ability to form a pentamer like IgM does, so their avidity is lower than IgM's. However, what they lack in avidity they gain in increased ability to squeeze through tight barriers like the blood-brain barrier or the placental barrier. An IgM pentamer couldn't do that, but the smaller monomers and dimers can. The new isotypes are all smaller finished products, which allows access to more places than IgM can go. In addition, although IgG, IgA, and IgE don't have the avidity that a pentamer of IgM has, the process of affinity maturation produces an antibody that has a much higher affinity. So in summary, isotype-switching and somatic hypermutation create a smaller but more agile and powerful antibody.

Cell Lineage

Plasma cells lose their surface proteins. Their job is no longer to LOOK for antigen (they don't need the surface proteins). Their job is to **produce antibodies**. Whether it was the first time encountering an antigen, where the naive B cell proliferates and differentiates into a plasma cell and secretes IgM, or it's a re-exposure where the memory B cell proliferates and differentiates into a plasma cell and secretes IgG, the outcome is the same: antibodies. Plasma cells are short-lived, produce massive numbers of antibodies, and are the central cells of adaptive immunity.

Memory cells retain their surface proteins. Their job is NOT to fight antigen. Their job is to stay alive for a long time (years, decades) waiting for that antigen to show its face again. When it does, the response is faster and more robust, and produces a proliferation of hyperspecialized plasma cells without

the need for a germinal center, affinity maturation, or isotype switching. The memory cell proliferates to make more memory cells and to produce plasma cells. Because it is already isotype-switched and affinity-matured, its proliferation of plasma cells and their production of antibodies is **faster** and **more robust** than the original response.

T-Cell-Independent B-Cell Activation (Honorable Mention)

Some B cells have the ability to express multiple surface IgM molecules. These multiple IgM molecules on one cell bind to large lipids and polysaccharides. This cross-linking of surface IgM receptors leads to T-cell-independent activation. T-cell-independent means no antigen-presenting cell, no cytokines, no class-switching. It just does what it does without a T cell. This means the antigen itself (lipid or polysaccharide) activates the B cell. Because there is no T cell involved in this process and the T cell is what signals the B cell to isotype-switch, the only antibody that is released is IgM. Without the signal to isotype-switch, the activated B cell does the only thing it knows how to do, which is to make IgM. This is a **weak**, **nonspecific** immune response, but requires no other intact cell lines to work.